PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MERCK & CO. INC.
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RAHWAY, NJ 07065-0907

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 SEP 2006
Applicant's or agent's file reference PCT 21394Y	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US04/32505	04 October 2004 (04.10.2004)	08 October 2003 (08.10.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC(8): A01K 67/00(2006.01),67/027(2006.01),67/033(2006.01)
USPC: 800/13,14,18

Applicant

MERCK & CO., INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☒ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☒ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	10 August 2006 (10.08.2006)	Anne-Marie Falk, Ph.D. Telephone No. (571)272-1600

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/32505

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 5,6,11 and 12

because:

☐ the said international application, or the said claim Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 5,6,11 and 12 are so unclear that no meaningful opinion could be formed *(specify)*:

Please See Continuation Sheet

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☐ no international search report has been established for said claims Nos. _____

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE
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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-4 and 7-10	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-4 and 7-10	NO
Industrial applicability (IA)	Claims 1-4 and 7-10	YES
	Claims NONE	NO

2. Citations and explanations:

Please see continuation sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/32505

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 5 and 6 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 5 and 6 are indefinite for the following reason(s):

Claims 5 and 6 recite the limitation that the "human B1 bradykinin gene is operatively fused to the native bradykinin B1 receptor protein." However, a gene cannot be fused to a protein and therefore the claims are indefinite. The claims will not be further treated on the merits.

Claims 11 and 12 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 11 and 12 are indefinite for the following reason(s):

Claims 11 and 12 recite the limitation "wherein the floxed gene has been excised." However, Claim 7, from which Claims 11 and 12 ultimately depend, requires that the animal must contain "a floxed marker gene." Since the limitations of the independent claim are necessarily incorporated into the dependent claims, Claims 11 and 12 likewise require the presence of "a floxed marker gene" with the further limitation "wherein the floxed gene has been excised." The animal cannot contain a floxed marker gene when the floxed gene has been excised. Thus, the limitation of the dependent claims conflicts with the limitations of the independent claim (Claim 7). The claims are indefinite and will not be further treated on the merits.

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

Section III. Non-establishment of opinion (description/claims/drawings unclear)

Claims 5 and 6 recite the limitation that the "human B1 bradykinin gene is operatively fused to the native bradykinin B1 receptor protein." However, a gene cannot be fused to a protein and therefore the claims are indefinite. The claims will not be further treated on the merits.

Claims 11 and 12 recite the limitation "wherein the floxed gene has been excised." However, Claim 7, from which Claims 11 and 12 ultimately depend, requires that the animal must contain "a floxed marker gene." Since the limitations of the independent claim are necessarily incorporated into the dependent claims, Claims 11 and 12 likewise require the presence of "a floxed marker gene" with the further limitation "wherein the floxed gene has been excised." The animal cannot contain a floxed marker gene when the floxed gene has been excised. Thus, the limitation of the dependent claims conflicts with the limitations of the independent claim (Claim 7). The claims are indefinite and will not be further treated on the merits.

V. 2. Citations and Explanations:

Claims 1-4 lack an inventive step under PCT Article 33(3) as being obvious over Pesquero et al. (2000), Hess et al. (1996), GenBank Accession No. BC034705 (July 2002), Menke et al. (1994), GenBank Accession No. NM_007539 (January 2002), Pesquero et al. (1996), and Bonaventure et al. (1999).

Pesquero et al. (2000) disclose a bradykinin B1 receptor knockout mouse. The mouse B1-receptor gene was cloned from a mouse genomic library and a targeting vector comprising a 1.0-kb genomic fragment 5' of the B1 coding region and a 7.0-kb genomic fragment 3' of the B1 coding region.

Hess et al. (1996) disclose that the agonist selectivity of the mouse B1 receptor differs significantly from the agonist selectivity of the human B1 receptor. The reference further discloses the isolation of a genomic clone encoding the mouse bradykinin B1 receptor.

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

GenBank Accession No. BC034705 (July 2002) discloses the cDNA sequence encoding the human bradykinin B1 receptor.

Menke et al. (1994) disclose the isolation of a cDNA clone encoding the human bradykinin B1 receptor using an expression cloning strategy.

GenBank Accession No. NM_007539 (January 2002) discloses the cDNA sequence encoding the mouse bradykinin B1 receptor.

Pesquero et al. (1996) disclose the cloning and functional characterization of the mouse bradykinin B1 gene. The gene encoding the mouse bradykinin B1 receptor was cloned from a mouse 129/SvJ genomic library by screening with a human B1 cDNA probe. The mouse bradykinin B1 receptor protein sequence is disclosed in Figure 1 and the cDNA sequence is disclosed in GenBank Accession No. NM_007539, as set forth above.

Bonaventure et al. (1999) disclose a knock-in mouse generated by replacing the coding region of the mouse 5-hydroxytryptamine $(5\text{-HT})_{1B}$ receptor gene with the coding region for the human 5-HT_{1B} receptor gene using homologous recombination in embryonic stem cells. The human coding sequence was thereby placed under control of the mouse 5-HT_{1B} receptor regulatory region and the expression pattern for the human receptor in the transgenic mouse was identical to the expression pattern of the mouse receptor in a wild-type mouse. The mouse gene is said to be 'humanized' by replacement with the human gene.

In view of the disclosure of Hess et al. (1996) noting the pharmacological differences between the human and mouse bradykinin B1 receptors, one of skill in the art would have been motivated to produce a humanized system for *in vivo* analysis of the pharmacology of the human bradykinin B1 receptor. Thus, the skilled artisan would have been motivated to generate a mouse that does not express the mouse bradykinin B1 receptor, but which instead expresses the human form in its place. Since knock-in technology was well known and well developed in the art, as demonstrated by Bonaventure et al., it would have been obvious to one of skill in the art, at the time of the invention, to have made a knock-in mouse by replacing the coding sequence of the mouse bradykinin B1 receptor gene with the human bradykinin B1 receptor gene so that the human gene would be placed under control of the endogenous mouse bradykinin B1 receptor regulatory region (i.e., promoter and other elements). Placing the human gene under control of the mouse endogenous elements would ensure an appropriate pattern of expression and appropriate levels of expression of the human bradykinin B1 receptor in mouse tissues. The skilled artisan would have anticipated a reasonable expectation of success in generating the knock-in mouse because all the necessary genomic fragments and coding sequences were readily available in the prior art, as discussed above, such that only routine experimentation would be required to generate the requisite targeting constructs and produce a knock-in mouse using gene targeting techniques that are well known and well developed in the art.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 7-10 lack an inventive step under PCT Article 33(3) as being obvious over Pesquero et al. (2000), Hess et al. (1996), GenBank Accession No. BC034705 (July 2002), Menke et al. (1994), GenBank Accession No. NM_007539 (January 2002), Pesquero et al. (1996), Bonaventure et al. (1999), and Milstone et al. (1999).

Pesquero et al. (2000) disclose a bradykinin B1 receptor knockout mouse. The mouse B1-receptor gene was cloned from a mouse genomic library and a targeting vector comprising a 1.0-kb genomic fragment 5' of the B1 coding region and a 7.0-kb genomic fragment 3' of the B1 coding region.

Hess et al. (1996) disclose that the agonist selectivity of the mouse B1 receptor differs significantly from the agonist selectivity of the human B1 receptor. The reference further discloses the isolation of a genomic clone encoding the mouse bradykinin B1 receptor.

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

bradykinin B1 receptor.

Pesquero et al. (1996) disclose the cloning and functional characterization of the mouse bradykinin B1 gene. The gene encoding the mouse bradykinin B1 receptor was cloned from a mouse 129/SvJ genomic library by screening with a human B1 cDNA probe. The mouse bradykinin B1 receptor protein sequence is disclosed in Figure 1 and the cDNA sequence is disclosed in GenBank Accession No. NM_007539, as set forth above.

Bonaventure et al. (1999) disclose a knock-in mouse generated by replacing the coding region of the mouse 5-hydroxytryptamine $(5\text{-HT})_{1B}$ receptor gene with the coding region for the human 5-HT_{1B} receptor gene using homologous recombination in embryonic stem cells. The human coding sequence was thereby placed under control of the mouse 5-HT_{1B} receptor regulatory region and the expression pattern for the human receptor in the transgenic mouse was identical to the expression pattern of the mouse receptor in a wild-type mouse. The mouse gene is said to be 'humanized' by replacement with the human gene.

Milstone et al. (1999) disclose that retained selection markers can affect neighboring genes and alter phenotypes in transgenic mice (page 1, column 1, paragraph 2 and abstract) and that removing selection markers after making one mutation allows for the use of the same selection marker in making additional mutations in the genome. The reference further discloses that these effects can be avoided by designing the targeting construct with loxP recombination sites flanking the marker gene (i.e., a floxed marker gene). The marker gene can then be excised from the mouse genome upon expression of Cre recombinase.

In view of the disclosure of Hess et al. (1996) noting the pharmacological differences between the human and mouse bradykinin B1 receptors, one of skill in the art would have been motivated to produce a humanized system for *in vivo* analysis of the pharmacology of the human bradykinin B1 receptor. Thus, the skilled artisan would have been motivated to generate a mouse that does not express the mouse bradykinin B1 receptor, but which instead expresses the human form in its place. Since knock-in technology was well known and well developed in the art, as demonstrated by Bonaventure et al. (1999), it would have been obvious to one of skill in the art, at the time of the invention, to have made a knock-in mouse by replacing the coding sequence of the mouse bradykinin B1 receptor gene with the human bradykinin B1 receptor gene so that the human gene would be placed under control of the endogenous mouse bradykinin B1 receptor regulatory region (i.e., promoter and other elements). Placing the human gene under control of the mouse endogenous elements would ensure an appropriate pattern of expression and appropriate levels of expression of the human bradykinin B1 receptor in mouse tissues. In view of the guidance of Milstone et al. (1999), the skilled artisan would have designed the targeting construct to include a floxed marker gene so that the selection marker could later be excised from the genome to avoid the effect on expression of neighboring genes and confounding phenotypes that accompany these undesired alterations in gene expression. The skilled artisan would have anticipated a reasonable expectation of success in generating the knock-in mouse because all the necessary genomic fragments and coding sequences were readily available in the prior art, as discussed above, such that only routine experimentation would be required to generate the requisite targeting constructs and produce a knock-in mouse using gene targeting techniques that are well known and well developed in the art.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1-4 and 7-10 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.